

# Blueprint Genetics Variant Classification Scheme for Dominant Monogenic Disorders

CLASSIFICATION	CATEGORY	CRITERIA
PATHOGENIC	1 Point Needed	1. Well-established mutation and wide consensus in the field on pathogenicity of the mutation. (Typically significant family segregation has been established and several publications support pathogenicity). – <b>1 Point</b> 2. In the setting of a novel disease in the family, <i>de novo</i> truncating variant absent from control populations in a gene where loss-of gene function has been established as a mechanism of pathogenicity for patient's disease (both paternity and maternity confirmed). – <b>1 Point</b> 3. LOF variant seen in at least 3 patients with the same phenotype (one of which can be the current patient) with cautious interpretation of the variants located in the last exon or in the last 50 base pairs of the penultimate exon – <b>1 Point</b>
	OR	<b>COMPULSORY A OR B:</b>
	5 Points Needed	A) Positive segregation with the disease ( $\geq 2$ families) and at least 5 unrelated patients (one of which can be the current patient) with same variant and phenotype. – <b>2 Points</b> OR B) $\geq 5$ cases (one of which can be the current patient) with same variant and phenotype reported. – <b>1 Point</b> <b>ADDITIONAL POINTS:</b> 1. Variant is novel or very rare in control populations (cannot be applied for ethnic backgrounds absent from control populations). – <b>1 Point</b> 2. Loss of gene function has been established as a mechanism of pathogenicity; scientific evidence for genotype – phenotype association exists. – <b>1 Point</b> 3. A missense variant predicted deleterious by majority of <i>in silico</i> tools applied and/or well-established paralogue mutation exists. – <b>1 Point</b> 4. <i>De novo</i> variant in the setting of a novel disease in the family (paternity and maternity unconfirmed). – <b>1 Point</b> 5. Variants considered deleterious (a substitution or indel in consensus splice site (+/-1, 2), nonsense and frameshift variants). – <b>1 Point</b> 6. Deficient protein function in appropriate functional assay(s), e.g. an animal model with equivalent mutation or splice site defect confirmed on mRNA level. – <b>1 Point</b> 7. Well-characterized other mutation at the same codon or same splice consensus site (+/-1, 2). – <b>1 Point</b> 8. Other strong data supporting pathogenic classification. – <b>1 Point</b>
LIKELY PATHOGENIC	2 Points Needed	1. Alterations resulting in premature truncation (e.g. frameshift, nonsense or consensus splice site (+/-1, 2) in a gene where loss-of gene function has been established as a mechanism of pathogenicity for patient's disease. – <b>1 Point</b> 2. Variant is novel or very rare in control populations (cannot be applied for ethnic backgrounds absent from control populations). – <b>1 Point</b>
	OR	
	4 Points Needed	1. Clear genotype phenotype correlation exist (e.g. MfS and <i>FBN1</i> ) – <b>1 Point</b> 2. Variant is novel or very rare in control populations (cannot be applied for ethnic backgrounds absent from control populations). – <b>1 Point</b> 3. Missense variant predicted deleterious by majority of <i>in silico</i> tools applied. – <b>1 Point</b> 4. A variant predicted to have an effect to the splicing by majority of <i>in silico</i> tools applied. – <b>1 Point</b> 5. An inframe deletion affecting conserved aa in a functional domain. – <b>1 Point</b> 6. Variant has been identified in $\geq 2$ individuals (one of which can be the current patient) with same disease manifestation. – <b>1 Point</b> 7. Evidence of a well-established paralogue mutation exists. – <b>1 Point</b> 8. <i>De novo</i> alteration in the setting of a novel disease in the family (paternity unconfirmed). – <b>1 Point</b> 9. Variants considered deleterious (a substitution or indel in consensus splice sites (+/-1, 2), nonsense and frameshift variants) identified in a gene with weak evidence for causativity in the disease type. – <b>1 Point</b> 10. Deficient protein function in appropriate functional assay(s), e.g. an animal model with equivalent mutation or splice site defect confirmed on mRNA level - <b>1 Point</b> 11. Well-characterized mutation at the same codon or same splice consensus site (+/-1, 2). – <b>1 Point</b> 12. Other strong data supporting pathogenic classification. – <b>1 Point</b>
Variant of unknown significance (VUS)		Variant has characteristics of being independent disease-causing mutation, however, insufficient or conflicting evidence exists.
LIKELY BENIGN	1 Point Needed	1. Control population minor allele frequency (1000G, ESP, SiSu, ExAC) is considerable (MAF>0.001) - (disease prevalence must be taken into account). – <b>1 Point</b>
	OR	
	2 Points Needed	1. MAF <0.001 in control populations but variant is detected in healthy controls with no disease association in a case-control study/studies. – <b>1 Point</b> 2. Homozygous variant in a gene with no association to the disease. – <b>1 Point</b> 3. Co-occurrence with a pathogenic mutation in the same gene (phase unknown) or in another gene that clearly explains the proband's phenotype. – <b>1 Point</b> 4. Majority of the <i>in silico</i> tools predict the substitution to be benign. – <b>1 Point</b> 5. Intact protein function observed in appropriate functional assay(s), e.g. splice region variant without abnormal splicing. – <b>1 Point</b> 6. Other data supporting benign classification. – <b>1 Point</b>
BENIGN	2 Points Needed	1. Does not segregate with the disease in family/ies with 2 or more affected individuals. – <b>1 Point</b> 2. Any additional criteria described below. – <b>1 Point</b>
	OR	
	4 Points Needed	1. Control population minor allele frequency (1000G, ESP, SiSu, ExAC) is considerable (MAF>0.001) - (prevalence of the disease must be taken into account). – <b>1 Point</b> 2. Homozygous variant in a gene with no association to the disease. – <b>1 Point</b> 3. Intact protein function observed in appropriate functional assay(s), e.g. splice region variant without abnormal splicing. – <b>1 Point</b> 4. Co-occurrence with a pathogenic mutation in the same gene (phase unknown) or in another gene that clearly explains the proband's phenotype. – <b>1 Point</b> 5. No disease association in small case-control study. – <b>1 Point</b> 6. Majority of the <i>in silico</i> tools predict the substitution to be benign. – <b>1 Point</b> 7. Other data supporting benign classification. – <b>1 Point</b>

**Disclaimers:**

- Every case is examined by our team in the light of the literature, publicly available clinical databases and the BpG in-house mutation database. Exceptions to the scheme can be made in complex cases or in the setting of poorly described patient phenotype.
- This classification scheme is not designed for the interpretation of variants considered as genetic modifiers or alleles predisposing to a disease with low-risk. Several variants classified with this scheme as likely benign or benign could function as disease modifiers. Classification as disease modifier can be applied when adequate scientific evidence has been established for a variant.
- It is not optimal for interpretation of alterations confounded by incomplete penetrance, variable expressivity, recessive inheritance, oligogenic inheritance, or skewed X-inactivation.
- Final classifications are subject to review and approval by Blueprint Genetics clinical staff and may differ from those predicted by the scheme.



# Blueprint Genetics Variant Classification Scheme for Recessive Monogenic Disorders

CLASSIFICATION	CATEGORY	CRITERIA
PATHOGENIC	1 Point Needed	1. Well-established variant and wide consensus in the field on pathogenicity of the variant. - <b>1 Point</b> <b>OR</b> 2. ≥2 cases (one of which can be the current patient) with the same phenotype and the same truncating variant (nonsense, frameshift, consensus splice site (+/-1, 2), start codon): in one patient observed in homozygous or compound heterozygous state (confirmed in <i>trans</i> ) and in one patient observed together with another disease-causing variant in the same gene (phase not determined) <b>AND</b> Variant frequency in control populations is consistent with an autosomal recessive disorder <b>AND</b> Loss of gene function is a well-established disease mechanism - <b>1 Point</b>
	OR	<b>COMPULSORY A OR B:</b>
	4 Points Needed	<b>A)</b> ≥3 cases (one of which can be the current patient) with the same phenotype and the same non-truncating variant (missense, splice region): <ul style="list-style-type: none"> <li>In at least two patients the variant is observed in homozygous or compound heterozygous state with a disease-causing variant (confirmed in <i>trans</i>)</li> <li>One patient may be included where the variant is observed either in homozygous state or together with another disease-causing variant in the same gene (phase not determined) - <b>1 Point</b></li> </ul> <b>OR</b> <b>B)</b> ≥5 cases (one of which can be the current patient) with the same phenotype and the same non-truncating variant: <ul style="list-style-type: none"> <li>In at least four patients the variant is observed together with a well-established variant in the same gene (phase not determined)</li> <li>One patient may be included where the variant is observed either in homozygous state or together with another disease-causing variant in the same gene (phase not determined) - <b>1 Point</b></li> </ul> <b>ADDITIONAL POINTS:</b> <ol style="list-style-type: none"> <li>Clear gene-phenotype correlation exists (e.g. <i>USH2A</i> and <i>USH2</i>) - <b>1 Point</b></li> <li>Variant frequency in control populations is consistent with an autosomal recessive disorder - <b>1 Point</b></li> <li>A missense variant predicted deleterious by majority of <i>in silico</i> tools applied - <b>1 Point</b></li> <li>Deficient protein function in appropriate functional assay(s) - <b>1 Point</b></li> <li>Well-characterized other disease-causing variant at the same codon or same consensus splice site (+/-1, 2) - <b>1 Point</b></li> <li>Variant occurs in <i>trans</i> with a pathogenic or likely pathogenic variant in the same gene - <b>1 Point</b></li> <li><i>De novo</i> variant is observed together with pathogenic or likely pathogenic variant in the same gene - <b>1 Point</b></li> </ol>
LIKELY PATHOGENIC	2 Points Needed	1. Alterations resulting in premature truncation (e.g. frameshift, nonsense or consensus splice site (+/-1, 2), start codon) in a gene where loss-of gene function has been established as a mechanism of pathogenicity - <b>1 Point</b> <b>AND</b> 2. Variant frequency in control populations is consistent with an autosomal recessive disorder - <b>1 Point</b>
	OR	1. Clear gene-phenotype correlation exists (e.g. <i>USH2A</i> and <i>USH2</i> ) - <b>1 Point</b> 2. Variant frequency in control populations is consistent with an autosomal recessive disorder - <b>1 Point</b> 3. Missense variant predicted deleterious by majority of <i>in silico</i> tools applied - <b>1 Point</b>
	4 Points Needed	4. ≥2 cases (one of which can be the current patient) with the same phenotype and the same non-truncating variant (missense, splice region): in one patient observed in homozygous or compound heterozygous state (confirmed in <i>trans</i> ) and in one patient observed either in homozygous state or together with a pathogenic or likely pathogenic variant in the same gene (phase not determined) <b>OR</b> Variant has been reported in <i>trans</i> with a pathogenic or likely pathogenic variant in the same gene <b>OR</b> ≥3 cases (one of which can be the current patient) with the same phenotype and the same non-truncating variant together with a well-established variant in the same gene (phase not determined) - <b>1 Point</b> 5. Variant occurs in <i>trans</i> with a pathogenic or likely pathogenic variant in the same gene (Note: This requires testing of parents to determine phase or close proximity of the variants, when NGS data can be used to determine the phase) - <b>1 Point</b> 6. Deficient protein function in appropriate functional assay(s) - <b>1 Point</b> 7. Well-characterized other disease-causing variant at the same codon or same consensus splice site – <b>1 Point</b> 8. Other strong data supporting pathogenicity - <b>1 Point</b>
Variant of uncertain significance (VUS)	Variant has characteristics of being a disease-causing variant, however, insufficient or conflicting evidence exists.	
LIKELY BENIGN	1 Point Needed	The allele frequency of the variant or the number of individuals homozygous for the variant in gnomAD or other publicly available database is greater than expected for the disorder (the prevalence of the disease in the population and the fraction explained by the specific gene must be taken into consideration) – <b>1 Point</b>
BENIGN	1 Point Needed	The allele frequency of the variant in gnomAD or other publicly available database is more than 5% - <b>1 Point</b>

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